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Our goal was to determine whether protein kinase C (PKC) activation caused a redistribution of PKC in smooth muscle. To accomplish this, we examined the three-dimensional distribution of PKC in smooth muscle before and after cell activation. Smooth muscle cells were enzymatically dispersed from the toad stomach. Bufo marinus, adhered to coverslips and treated with either 10 μM carbachol, 1 μM phorbol 12-myristate 13-acetate (PMA), 1 μM 4-α phorbol 12,13-didecanoate (4-α PMA, an inactive phorbol ester) or amphibian physiological saline. Cells were then fixed with paraformaldehyde, permeabilized with triton X-100 and immunofluorescently labelled for PKC and vinculin. Vinculin was labelled to identify the cell surface and cell membrane contractile filament anchorage sites. The anti-PKC IgM was capable of immunoprecipitating PKC from a PKC containing reaction mixture. The immunoprecipitation of PKC was found to increase in a dose-dependent manner by adding PMA to the reaction mixture. This indicated that the anti-PKC used for immunolocalization preferentially recognized the activated form of PKC. Fluorescent images of labelled cells were collected as a through focus series of two-dimensional images spaced at 0.25 μm intervals using a digital imaging microscope. These images were subjected to a deblurring image restoration program using a constrained deconvolution algorithm. The restored images were reconstructed into three-dimensional displays of the intracellular distribution of PKC and vinculin. At rest, PKC and vinculin were located at the cell periphery and organized longitudinally into roughly parallel strands. At the cell periphery, 18% of the PKC colocalized with vinculin. In the cell interior, labelling for PKC was sparse and diffuse. Following carbachol, PKC exhibited a more pronounced and strand-like cytosolic distribution. Also, there was an increase in the total fluorescence intensity of PKC labelling. Treatment with PMA but not 4-α PMA produced similar changes in PKC distribution. These results indicate that in the resting smooth muscle cell there is a pool of activated PKC near the cell membrane and that after stimulation the activated PKC is not confined to the cell membrane but can be observed throughout the cytosol. In the cytosol, the apparent association of PKC with contractile filaments suggests a role for PKC in modulatory contractile function.

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G-PROTEIN DEPENDENT ENHANCEMENT OF VASCULAR SMOOTH MUSCLE MYOFILAMENT Ca^{2+} SENSITIVITY

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In smooth muscle, force development is more sensitive to Ca^{2+} during agonist stimulation than during membrane depolarization. We used alpha-toxin permeabilized smooth muscle to investigate the mechanism(s) of the enhanced Ca^{2+} sensitivity. This preparation retains receptor-coupled events while allowing control of the intracellular environment. Stimulation of alpha-toxin permeabilized rabbit mesenteric arteries with 0.3 μM Ca^{2+} induced a monotonic increase in force and myosin light chain (MLC) phosphorylation. The inclusion of 10 μM norepinephrine (NE) and 10 μM GTP resulted in the monotonic increase in force to significantly greater levels. However, MLC phosphorylation levels only transiently increased to higher levels in response to NE plus GTP, then fell with time to values similar to those in response to Ca^{2+} alone. Exposure of the permeabilized arteries to inhibitors of protein kinase C (PKC) specifically abolished the enhanced force and transient increase in MLC phosphorylation in response to NE plus GTP. These data suggest that receptor dependent PKC activation inhibits MLC phosphatase activity, possibly by PKC catalyzed phosphorylation of a MLC phosphatase inhibitor. In addition, there must be a second process that maintains the agonist induced enhanced force at MLC phosphorylation levels similar to those in response to Ca^{2+} alone. This study was supported in part by NIH grants HL37956 and HL46704.

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TWO DISTINCT α_1-ADRENOCEPTOR SUBTYPES IN RABBIT THORACIC AORTA

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α_1-Adrenoceptors are not homogeneous in all tissues and their heterogeneity has been suggested to be related in part to the presence of different α_1-adrenoceptor subtypes. In the rabbit thoracic aorta, noradrenaline with a very wide range of concentrations (from 1 nM to 100 μM) produced a concentration-dependent contraction. The concentration-response curve was antagonized by prazosin, resulting in a rightward displacement of the curve. However, the shift was not proportional to the concentration of prazosin; Schild plots showed that the inhibition by prazosin was biphasic, implying that noradrenaline acted through two receptor populations (pK_B = 10.0 and 8.8). However, in the strips pretreated with chloroethylclonidine (CEC; an α_{1B} inactivating agent), noradrenaline with a narrow range of concentrations (from 30 nM to 100 μM) produced a concentration-response curve, which was competitively inhibited by prazosin (pK_B = 8.3). ^{3}H-prazosin bound to two distinct populations of α_1-adrenoceptors of the aortic membranes (pK_D = 9.94 and 8.59). Pretreatment with CEC abolished the 3H-prazosin binding to the prazosin-high but not -low affinity sites. Noradrenaline showed slightly higher affinity for the prazosin-high affinity site than for the low affinity site. From these results, it is suggested that in the rabbit thoracic aorta there are two distinct α_1-adrenoceptor subtypes

(presumably α_{1B} and α_{1L} subtypes), both of which are involved in the noradrenaline-induced contraction.

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MINOR ROLE FOR DIRECT ADRENOCEPTOR-MEDIATED CALCIUM ENTRY IN RAT MESENTERIC RESISTANCE ARTERIES

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Ca^{2+}-entry into vascular smooth muscle cells occurs through potential-dependent, dihydropyridine-sensitive calcium channels, and possibly also through calcium channels which are activated by receptor activation, independent of membrane potential (ROCs). We have investigated the possible functional role of adrenoceptor-mediated ROCs in rat mesenteric small arteries from Wistar rats. $[Ca^{2+}]_i$ determined by fura-2 fluorescence and force were measured simultaneously using a myograph. Depolarization with high-potassium solution (K-PSS) elevated $[Ca^{2+}]_i$ and induced contraction. Further addition of 10 µM noradrenaline (NA) did not elevate $[Ca^{2+}]_i$ further, but enhanced tone, thus confirming that agonist activation causes calcium-sensitization. When this protocol was repeated in the presence of calcium channel inhibitors (felodipine (1 nM) or D-600 (1 µM)), K-PSS induced neither maintained tone nor maintained elevation of $[Ca^{2+}]_i$, but the further addition of NA still caused a rise in tone and $[Ca^{2+}]_i$, to about half the previous levels. Repetition of the protocol in the presence both of calcium channel inhibitors and also drugs which deplete intracellular calcium stores (ryanodine (10 µM) or thapsigargin (1 µM)), showed that the NA-induced rise in $[Ca^{2+}]_i$ was inhibited, but a small (10-20% of control) rise in tone remained. The experiments suggest that ROCs do not contribute substantially to control of $[Ca^{2+}]_i$, and that effects of NA on tone which are independent of membrane potential are due primarily to calcium-sensitization but also to release of Ca^{2+} from intracellular stores.

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MODE OF EXISTENCE OF 74 KDA PROTEIN, ONE OF THE GELSOLIN FAMILY IN DEVELOPMENTAL STAGE OF VASCULAR SMOOTH MUSCLE

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The 74 KDa protein of the gelsolin family is distributed in non-muscle cells with exocytic function, but not in normal adult smooth muscle cells. In primary culture of smooth muscle from bovine carotid artery, we found that this protein was gradually expressed, arriving at maximum in confluent state by immunoblotting method. On the other hand, in the immunofluorescent method of the cultured cells 74 Kda protein was not expressed in all the cells with deformed globular feature on the 1st day without serum, but smooth muscle type of alpha-actin and smooth muscle myosin were clearly expressed. On 4th day after addition of serum, 74 KDa protein was expressed at the surface membrane of all the globular cells of which numbers were slightly increased by slow cell division. At time bipolar cells appeared underneath the globular cells. These cells had no 74 Kda protein and alpha-actin. Once bipolar cells appeared, their growth rate was very fast and their numbers increased rapidly. When cells were proceeding to confluent state, accompanied by hills and valleys structure, globular cells were gathered, and formed tight round structure on the top of the hills. At that time alpha-actin began to be expressed in abundant bipolar cells.

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CORONARY VASODILATOR RESPONSE TO BRADYKININ: ROLE OF CYTOCHROME P450 AND K^+ CONDUCTANCE

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The rat coronary vasodilator response to bradykinin (BK) is reportedly independent of NO and cyclooxygenase and lipoxygenase products of arachidonic acid (AA). We investigated the role of cytochrome P450 (P450) in the coronary vasodilator response to BK using the Langendorff heart preparation which was preconstricted with nitroarginine (50 µM) to eliminate NO. As reported, neither nitroarginine nor indomethacin (2.8 µM) affected the coronary vasodilator